

Figure 1/7

Nucleotide Sequence of wtGFP (Chalfie et al. Science. (1994). 263. 802-5):

SEQ ID No.1

atg agt aaa gga gaa gaa ctt ttc act gga gtt gtc cca att ctt gtt 48
gaa tta gat ggt gat gtt aat ggg cac aaa ttt tct gtc agt gga gag 96
ggt gaa ggt gat gca aca tac gga aaa ctt acc ctt aaa ttt att tgc 144
act act gga aaa cta cct gtt cca tgg cca aca ctt gtc act act ttc 192
tct tat ggt gtt caa tgc ttt tca aga tac cca gat cat atg aaa cgg 240
cat gac ttt ttc aag agt gcc atg ccc gaa ggt tat gta cag gaa aga 288
act ata ttt ttc aaa gat gac ggg aac tac aag aca cgt gct gaa gtc 336
aag ttt gaa ggt gat acc ctt gtt aat aga atc gag tta aaa ggt att 384
gat ttt aaa gaa gat gga aac att ctt gga cac aaa ttg gaa tac aac 432
tat aac tca cac aat gta tac atc atg gca gac aaa caa aag aat gga 480
atc aaa gtt aac ttc aaa att aga cac aac att gaa gat gga agc gtt 528
caa cta gca gac cat tat caa caa aat act cca att ggc gat ggc cct 576
gtc ctt tta cca gac aac cat tac ctg tcc aca caa tct gcc ctt tcg 624
aaa gat ccc aac gaa aag aga gac cac atg gtc ctt ctt gag ttt gta 672
aca gct gct ggg att aca cat ggc atg gat gaa cta tac aaa tag 717

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Amino Acid Sequence of wtGFP (Chalfie et al. Science, (1994). 263. 802-5

SEQ ID No.2

Met	Ser	Lys	Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	1	5	10	15
Glu	Leu	Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	20	25	30	
Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	35	40	45	
Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Phe	50	55	60	
Ser	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys	Arg	65	70	75	80
His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	85	90	95	
Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	100	105	110	
Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	115	120	125	
Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	130	135	140	
Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met	Ala	Asp	Lys	Gln	Lys	Asn	Gly	145	150	155	160
Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Ser	Val	165	170	175	
Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro	180	185	190	
Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser	195	200	205	
Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val	Leu	Leu	Glu	Phe	Val	210	215	220	
Thr	Ala	Ala	Gly	Ile	Thr	His	Gly	Met	Asp	Glu	Leu	Tyr	Lys			225	230	235	

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Predicted Amino Acid Sequence of F64L-S175G-E222G-GFP:

SEQ ID No.3

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Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
1      5      10      15
Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
20      25      30
Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
35      40      45
Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu
50      55      60
Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Arg
65      70      75      80
His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
85      90      95
Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
100     105     110
Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
115     120     125
Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
130     135     140
Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
145     150     155     160
Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Gly Val
165     170     175
Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
180     185     190
Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
195     200     205
Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Gly Phe Val
210     215     220
Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
225     230     235

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Predicted Amino Acid Sequence of F64L-S65T-S175G-GFP:

SEQ ID No.4

Met	Ser	Lys	Gly	Glu	Glu	Leu	Phe	Thr	Gly	Val	Val	Pro	Ile	Leu	Val	1	5	10	15
Glu	Leu	Asp	Gly	Asp	Val	Asn	Gly	His	Lys	Phe	Ser	Val	Ser	Gly	Glu	20	25	30	
Gly	Glu	Gly	Asp	Ala	Thr	Tyr	Gly	Lys	Leu	Thr	Leu	Lys	Phe	Ile	Cys	35	40	45	
Thr	Thr	Gly	Lys	Leu	Pro	Val	Pro	Trp	Pro	Thr	Leu	Val	Thr	Thr	Leu	50	55	60	
Thr	Tyr	Gly	Val	Gln	Cys	Phe	Ser	Arg	Tyr	Pro	Asp	His	Met	Lys	Arg	65	70	75	80
His	Asp	Phe	Phe	Lys	Ser	Ala	Met	Pro	Glu	Gly	Tyr	Val	Gln	Glu	Arg	85	90	95	
Thr	Ile	Phe	Phe	Lys	Asp	Asp	Gly	Asn	Tyr	Lys	Thr	Arg	Ala	Glu	Val	100	105	110	
Lys	Phe	Glu	Gly	Asp	Thr	Leu	Val	Asn	Arg	Ile	Glu	Leu	Lys	Gly	Ile	115	120	125	
Asp	Phe	Lys	Glu	Asp	Gly	Asn	Ile	Leu	Gly	His	Lys	Leu	Glu	Tyr	Asn	130	135	140	
Tyr	Asn	Ser	His	Asn	Val	Tyr	Ile	Met	Ala	Asp	Lys	Gln	Lys	Asn	Gly	145	150	155	160
Ile	Lys	Val	Asn	Phe	Lys	Ile	Arg	His	Asn	Ile	Glu	Asp	Gly	Gly	Val	165	170	175	
Gln	Leu	Ala	Asp	His	Tyr	Gln	Gln	Asn	Thr	Pro	Ile	Gly	Asp	Gly	Pro	180	185	190	
Val	Leu	Leu	Pro	Asp	Asn	His	Tyr	Leu	Ser	Thr	Gln	Ser	Ala	Leu	Ser	195	200	205	
Lys	Asp	Pro	Asn	Glu	Lys	Arg	Asp	His	Met	Val	Leu	Leu	Glu	Phe	Val	210	215	220	
Thr	Ala	Ala	Gly	Ile	Thr	His	Gly	Met	Asp	Glu	Leu	Tyr	Lys			225	230	235	

Figure 5/7

Flow cytometry of GFP mutations

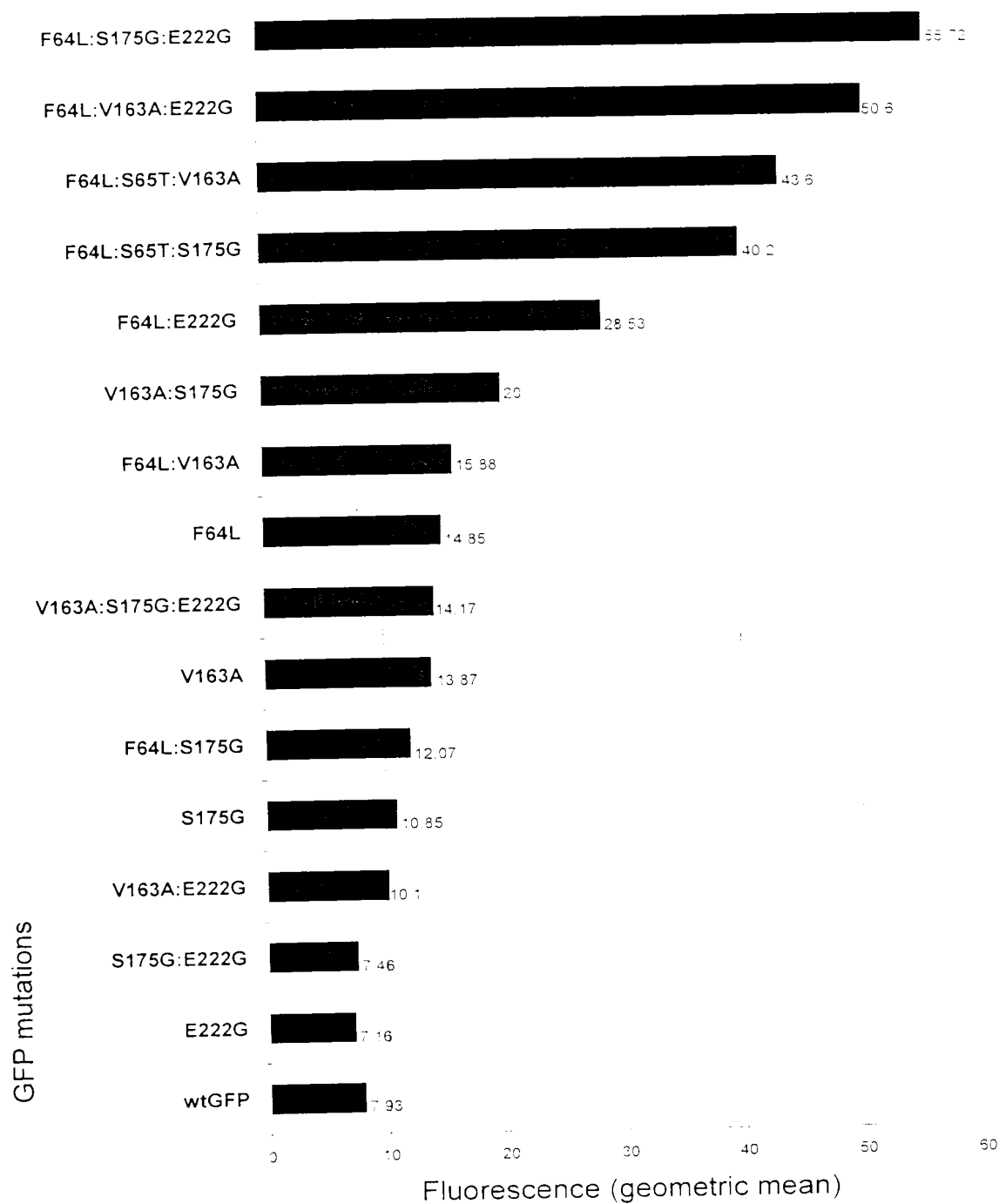


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Photobleaching of GFP mutations

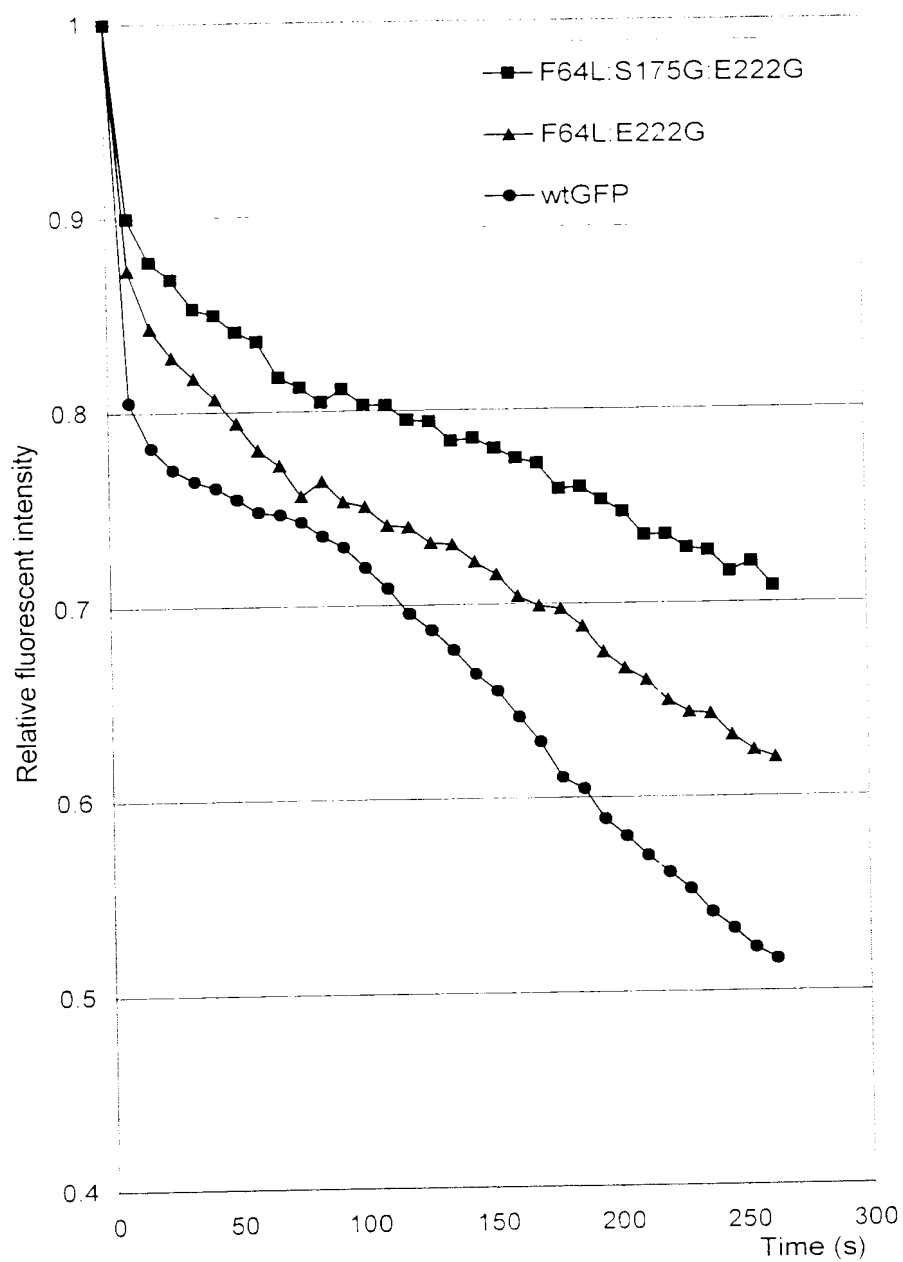


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Live Cell CHO-HIR NF κ B Assay
P65-tri GFP Assay T = 30 mins

